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IDENTIFICATION OF GENETIC CHARACTERISTICS OF E. COLI RESISTANT TO ANTIMICROBIALS

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Identification of Genetic Characteristics of *E. coli* Resistant to Antimicrobials

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Abstract

Antimicrobial resistance (AMR) has become a major concern in veterinary and public health. Surveillance programs such as the Canadian Integrated Program for Antimicrobial Surveillance (CIPARS) determine the prevalence of AMR in bacteria from animals, including the intestinal bacterium *Escherichia coli*. This helps to assess the overall situation of AMR in animals. However, a variety of AMR genes can encode the same resistance and knowing which genes are present in bacteria of different origins or within the same population but at different times helps us to understand better the epidemiology of AMR, how AMR genes can move between bacteria, and may provide evidence for changes not identifiable on the basis of antimicrobial susceptibility testing alone. In addition, approaches based on statistical associations between genes allow us to understand which AMR genes are linked and move together between bacteria, and how the use of one antimicrobial agent may select for multiple resistances.

This project investigated the distribution of antimicrobial resistance genes in *E. coli* from cattle presented to slaughter isolated by CIPARS between 2003 and 2007 in Western Canada in order to provide a baseline for the frequency of the major AMR genes and a comparison basis with *E. coli* from other origins. Associations between genes were also identified, which point to gene linkages and potential for co-selection.

Polymerase chain reaction was used to assess the distribution of major AMR genes for tetracycline, sulfonamides, streptomycin, ampicillin, trimethoprim, gentamicin, and chloramphenicol. Some similarities but also some differences were observed when comparing the results of the present study on cattle presented to slaughter and previous studies performed in Canada on cow-calf operations or on swine, thus suggesting that despite the frequent transfer of AMR genes across bacterial populations, some aspects of AMR epidemiology are specific to cattle. An imperfect correlation between antimicrobial susceptibility testing and presence of AMR genes were observed for some antimicrobials, which stress the need for AMR gene detection and changes in the breakpoints used for classification into resistant and susceptible for epidemiological purposes. Ampicillin resistance in generic *E. coli* from cattle seems not to be caused by the genes investigated here and further studies will be needed to identify which genes are responsible for this type of resistance. AMR gene associations already observed in *E. coli* of other origins were identified in this study, thus supporting the hypothesis of wide distribution of a few major mobile genetic elements across bacterial populations of diverse origins. A very few highly multiresistant *E. coli* isolates were shown to carry the extended-spectrum cephalosporin resistance gene bla_{CMY-2}. This gene encodes resistance

to ceftiofur and most other β-lactam antibiotics, is usually located on transferable plasmids, and seems to be associated with other AMR genes including the *floR* gene encoding resistance to florfenicol. Because of its significance for both animal and human health, the situation with this gene will need to be regularly monitored in the future.

Identification of Genetic Characteristics of *E. coli* Resistant to Antimicrobials (20060117)

Introduction

Antimicrobial resistance (AMR) is a major problem for animal and human health and the transfer of resistant bacteria between animals, as well as between animals and humans through the food chain has become a source of concern. This concern is increased by the potential for AMR genes to move between bacteria, including between harmless commensal bacteria such as *E. coli* selected through routine use of antimicrobials and animal or zoonotic pathogens. There is a particular concern with regard to genetic linkage of antimicrobial resistance determinants and co-selection of multiple AMR genes located either on the bacterial chromosome or on transferable genetic elements such as a plasmid through the use of single antimicrobials. Based on these concerns, surveillance programs have been setup to monitor the AMR situation in food animals in many countries, including in Canada with the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS). Since 2002, CIPARS has been monitoring the situation of AMR in swine, chicken, and cattle, conducting antimicrobial susceptibility testing on representative samples of generic *E. coli* and other bacteria from food animals over the whole country (Government Canada, 2007). Although these data provide a good picture of AMR prevalence in cattle presented to slaughter in Canada, they do not allow us to address appropriately the issue of AMR gene linkages and co-selection. Since a variety of different AMR genes may be hiding behind a simple resistance phenotype, genetic approaches may also provide more detailed information on the relationships between AMR in different populations than susceptibility testing results and may allow us to detect new emerging changes in AMR epidemiology not readily identifiable with antimicrobial susceptibility testing alone. They may also help to identify ecological compartmentalization in the distribution of AMR genes and to identify potential ways to control the spread of AMR. By evaluating the extent of gene linkage, we can provide the information necessary for policy makers to make rational decisions on the approval of new antimicrobials and the regulation of existing antimicrobials. Evidence of gene linkage is necessary to examine whether restricting the use of a single drug will be effective in decreasing resistance to that drug over time. The persistence of vancomycin-resistant enterococci in Danish swine populations after the ban of avoparcin (Bager et al., 1999) and the persistence of resistance to chloramphenicol in porcine *E. coli* in Canada (Travis et al., 2006) are prime examples of the effects of gene linkages and co-selection on the distribution of AMR. Thus, identifying the major AMR gene linkages may help to avoid co-selection and to control AMR by judicious use of selected antimicrobials. The beef industry is vital to the Canadian, and particularly the Saskatchewan economy, and proactive research is required to ensure the unhampered growth of the cattle industry in this province and to protect public health.

The first step in any control program is baseline information. To implement effective control and to evaluate the effectiveness of control programs it is essential to know exactly what hazards move along the chain, how they are transmitted through each stage and how one might effectively test for their presence. Extensive work has been done worldwide on the distribution of AMR genes in *E. coli* from food animals, particularly in

swine. However, only little is known about it in cattle, particularly cattle in Canada. To the best of our knowledge, only one extensive study has been published on this subject and it was targeted at cow-calf operations rather than cattle presented to slaughter (Gow et al., 2008). Thus, the present study was intended to fill a gap in our knowledge of AMR in cattle presented to slaughter in Canada and to provide a baseline for the distribution of AMR genes as well as some initial information on the major AMR gene linkages present in bacteria from this population.

CIPARS results show that the most frequent antimicrobial resistances in *E. coli* from cattle in Canada are to tetracycline, sulfonamides, aminoglycosides such as streptomycin, and β-lactams such as ampicillin and amoxicillin/clavulanate (Government Canada, 2007). Therefore, a major effort was put in this study to assess the distribution of AMR genes for these antimicrobials. Since integrons represent a major cause of multiresistance by their ability to bring together multiple AMR genes, we also assessed the prevalence of the *int1* gene for the integrase of class 1 integrons (Rowe-Magnus and Mazel, 2002). Finally, bla_{CMY-2}, which is a frequent gene for resistance to extended-spectrum cephalosporin in *Enterobacteriaceae* from animals and a major concern for public health was also investigated (Tragesser et al., 2006; Winokur et al., 2001).

Methods

Bacterial isolates. *E. coli* isolates collected by CIPARS from cecal samples of cattle presented to slaughter in British Columbia, Alberta, Saskatchewan, and Manitoba between 2003 and 2007 (n=275) were selected and used for the present study as follows. Both isolates resistant (n=109) to any of the antimicrobial agents tested by CIPARS (Government Canada, 2007) and isolates fully susceptible (n=166) to all the antimicrobial tested were used in this study. The antimicrobial agents tested in the susceptibility testing panel included tetracycline, sulfonamides, trimethoprim-sulfamethoxazole, ampicillin, amoxicillin-clavulanic acid, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, streptomycin, kanamycin, gentamicin, amikacin, nalidixic acid, and ciprofloxacin.

AMR gene detection. Multiplex PCR protocols for the detection of the following AMR genes previously developed and validated in our laboratory were used (Kozak and Boerlin unpublished).

- Sulfonamides: *sul1*, *sul2*, *sul3*
- Tetracycline: *tet*(A), *tet*(B), *tet*(C)
- β-lactams: bla_{CMY-2}, bla_{TEM}, bla_{SHV}
- Aminoglycosides: *aadA*, *strA/strB*, *aac(3)IV*
- Phenicols: *catA1*, *cmlA*, *floR*.

In addition, a PCR protocol from the literature for the *dfrA1* gene (trimethoprim resistance) was optimized and validated, and a new PCR protocol for the class 1 integrase gene *int1* was developed and validated for the purpose of this study. All lysate preparations using a previously described protocol (Miserez et al., 1998) and PCR setup were done using a Beckman Coulter liquid handling robot.

All frequency estimates and statistical tests (Fisher's exact and odds ratios) were obtained using the STATA9 software.

Results and Discussion

AMR gene frequencies: The overall genotyping results are presented in Table 1 and Figure 1. One or several of the resistance genes investigated was detected in 101 out of 109 resistant isolates (93%). Twenty-four of the susceptible isolates (14%) possessed one of the genes investigated. The most common resistance genes among isolates resistant to ampicillin, chloramphenicol, sulfonamides, streptomycin, and tetracycline were bla_{CMY-2}, *floR*, *sul2*, *strA/strB*, and *tet*(B), respectively. The bla_{TEM}, bla_{SHV}, and *aac(3)IV* genes were not detected. The *int1* gene was detected in 14 isolates and none of these isolates was susceptible to all the antimicrobials tested. These data provide a first baseline for the prevalence of AMR genes in isolates from cattle presented to slaughter. The tetracycline resistance gene prevalences among resistant isolates are similar to those found in cow-calf operations (Gow et al., 2008), with *tetB* being the most frequent one and *tetC* the least frequent. This predominance or high frequency of *tetB* seems to be a recurrent phenomenon across commensal *E. coli* populations from different animal species (Boerlin et al., 2005; Guerra et al., 2003; Gow et al., 2008). The same is true with *sul2* being the predominant sulfonamides resistance gene. Our results also show that the *sul3* gene, which is highly prevalent in porcine *E. coli* in Canada (Boerlin et al., 2005), is still rare in isolates from cattle. A comparison is difficult for streptomycin resistance, since not all the genes included here were used in previous studies, but our results show a strong predominance of *strA/strB* as a cause of streptomycin resistance in generic *E. coli* from cattle presented to slaughter. This is different from the situation in swine, in which *aadA* had been shown to be dominant (Boerlin et al., 2005). This also fits to the relatively low prevalence of class 1 integrons (*int1*) observed here, which are the major carrier of *aadA* gene cassettes. However, we don't have a definitive explanation for this difference between cattle presented to slaughter and swine at this stage, except that there may be a different use of spectinomycin in swine and cattle (the *aadA* gene encodes resistance to both streptomycin and spectinomycin, whereas *strA/strB* encode resistance to streptomycin only). The only β-lactam resistance gene detected here was the bla_{CMY-2} gene (in only two isolates). The low prevalence of this gene for extended-spectrum cephalosporin resistance is reassuring. However, because of the use of ceftiofur in cattle and strong potential for selection, its mere presence dictates caution and warrants further monitoring in the future. The number of resistant isolates does not allow clear-cut conclusions about the chloramphenicol resistance situation. However, our findings suggest that the situation may be quite different in cattle presented to slaughter (predominance of *floR*) than in cow-calf operations (predominance of *catI*) and in swine (predominance of *cmlA*). The epidemiology of chloramphenicol resistance in cattle may be more driven by the use of florfenicol than it is in other commodities such as swine, where it is probably driven by co-selection through other antimicrobials (Travis et al., 2006). Finally, the *dfrA1* gene, which is the most frequent trimethoprim resistance gene in cow-calf operations (Gow et al., 2008) was not detected among the only two isolates resistant to sulfonamide/trimethoprim in this study. Instead, sequencing of the variable region of the class 1 integron present in these two isolates showed that they both possessed a *dfrA12* gene (data not shown). However, because of the low numbers of isolates resistant to trimethoprim in this study this may be only an anecdotic observation.

Correlation phenotypes-genotypes: The overall agreement between presence/absence of the genes for resistance to specific antimicrobial agents and resistance/susceptibility to

each antimicrobial agent are presented in Tables 1 and 2. Some important disagreements were observed for tetracyclines, streptomycin, and ampicillin. For tetracycline, most of the disagreement was caused by the presence of a *tetC* gene in isolates classified as non-resistant, and for streptomycin by the presence of either *aadA* or *strA/strB* in isolates also classified as non-resistant. Recent work in our laboratory has shown that the *tetC* gene is associated with low tetracycline MICs and that isolates of various sources carrying this gene are frequently misclassified on the basis of susceptibility testing (Chalmers et al., submitted). The misclassification problem for streptomycin has also already been identified repeatedly and changes in epidemiological breakpoints have been suggested for this antimicrobial agent (Government Canada, 2005; Sunde et al., 2005). For ampicillin, the reverse was true, with the majority of disagreement caused by the absence of any of the three β-lactam resistance genes investigated in resistant isolates. Other β-lactam resistance genes than those investigated here are likely to be the source of these unexplained phenotypes and there is a need to identify them for the purpose of future monitoring.

AMR gene profiles and gene associations. The combination of resistance gene profiles observed in the collection of isolates tested is presented in Table 3 and the analysis of associations between pairs of AMR genes in Table 4. A relatively large number of AMR gene profiles were identified among the isolates tested and many of them have already been observed in other studies. The multitude of AMR genes found in the two bla_{CMY-2} positive isolates is noticeable and worrisome, particularly because of the presence of the *floR* gene in both of them, opening the door for the potential co-selection of extended-spectrum cephalosporin resistance through the use of florfenicol. However, because of the frequent presence of multiple resistance plasmids within a single *E. coli* isolate, these profiles may be misleading with regards to physical linkages of resistance genes on single mobile elements. Thus, pair wise associations may be more informative in this regard.

A large numbers of statistically significant AMR gene associations were identified in our isolate collection (Table 4). However, some caution is needed in their interpretation. Multiple comparisons may lead to the identification of spurious associations, and some others may be the result of clonal spread of strains rather than of the presence of linkage on mobile genetic elements. In our experience, the latter phenomenon is associated with very high odds ratios superior to 30 or 40. Several occurrences of such high odds ratios were found here, but unfortunately, the low number of isolates carrying some of the genes of interest did not allow us to calculate odds ratios in every instance and some interpretations remain difficult. Some associations based on known genetic elements such as integrons for *sul1*, *aadA*, and *int1* , or for *sul3* and *cmlA* are quite obvious (Rowe-Magnus and Mazel., 2002; Bischoff et al., 2005). Others, such as the positive association between sul2, *strA/strB*, and *tetB*, or the negative association between *tetA* and *tetB* have already been described in other populations (Boerlin et al., 2005; Gow et al., 2008; Maynard et al., 2003). Other ones may be new and of more concern, including those between bla_{CMY-2}, *floR*, *sul1*, *sul2*, *tetA*, and *int1*, because of the potential for co-selection of AMR genes with high significance for both animal and public health located on mobile genetic elements with genes with less significance coding for resistance to antimicrobial agents utilized in cattle populations.

Adoption of Project Results

In conclusion, this study has provided a first baseline for the distribution of AMR genes in cattle presented to slaughter from Western Canada, which will serve as a basis for future monitoring of the AMR situation in cattle and for comparison with other populations to better apprehend the epidemiology of AMR in bacteria from food animals and zoonotic agents. We have shown that some differences exist in the distribution of AMR genes between commodities (for instance for sulfonamide, streptomycin, and chloramphenicol resistance genes), that distinguish cattle presented to slaughter from others. We also found a few isolates carrying genes for extended-spectrum cephalosporin, which seem to be associated and linked to florfenicol resistance and to resistance to numerous other antimicrobials used in the cattle industry. This may become a source of concern if the selective pressure exerted by antimicrobial use leads to an increase in prevalence of types of resistance important for public health and further monitoring is needed to support intervention if needed. Further work is going on in our laboratory to confirm physical linkages between these genes with molecular approaches. Our results have identified and confirmed some problems with regards to susceptibility testing and breakpoint setting for epidemiological surveillance of AMR, which will need to be addressed in the future. Finally, our results show that β-lactam resistance in *E. coli* from cattle presented to slaughter may not be as frequently encoded by bla_{TEM} or bla_{SHV} genes as expected and that other genes not considered in this study are frequently encoded in *E. coli* from cattle. Further work is needed to identify them, to evaluate their significance, and to monitor them adequately in the future.

Tables and Figures

Table 1. Frequency of AMR genes detected among *E. coli* isolates resistant and susceptible to specific antimicrobial agents.

Antimicrobial agent	Gene	Resistant	Susceptible	Total
Tetracycline	*tet*(A)	33/97 (34.0%)	1/178 (0.5%)	34/275
	tet(B)	52/97 (53.6%)	2/178 (1.1%)	54/275
	tet(C)	14/97 (14.4%)	25/178 (14.0%)	39/275
Sulfonamides	*sul1*	9/38 (23.7%)	1/237 (0.4%)	10/275
	sul2	30/38 (78.9%)	0/237 (0.0%)	30/275
	sul3	1/38 (2.6%)	0/237 (0.0%)	1/275
Streptomycin	*aadA*	4/40 (10.0%)	8/235 (3.4%)	12/275
	strA/strB	36/40 (90.0%)	18/235 (7.7%)	54/275
Ampicillin	bla_{TEM}	0/18 (0.0%)	0/257 (0.0%)	0/275
	bla_{SHV}	0/18 (0.0%)	0/257 (0.0%)	0/257
	bla_{CMY-2}	2/18 (11.1%)	0/257 (0.0%)	2/257
Chloramphenicol	*catA1*	1/5 (20.0%)	0/270 (0.0%)	1/275
	cmlA	0/5 (0.0%)	1/270 (0.4%)	1/275
	floR	5/5 (100.0%)	0/270 (0.0%)	5/275
Trimethoprim	*dfrA1*	0/2 (0.0%)	0/273 (0.0%)	0/275

No resistance to gentamicin and no *aac(3)IV* gene were detected among the 275 isolates tested.

Table 2. Agreement between phenotypes and genotypes for resistance to specific antimicrobial agents.

Antimicrobial agent	Resistant		Susceptible		Overall agreement
	Gene present	Gene absent	Gene present	Gene absent	
Tetracycline	94	3	26	152	89.5%
Sulfonamides	37	1	1	236	99.3%
Streptomycin	38	2	26	209	90.0%
Ampicillin	2	16	0	257	94.2%
Chloramphenicol	5	0	1	269	99.6%
Gentamicin	0	0	0	275	100%
Trimethoprim	0	2	0	273	99.3%

The overall agreement was calculated as the proportion of total isolates with matching results between phenotype and genotype (i.e. resistant to an antimicrobial agent with one or more resistance gene(s) for this specific antimicrobial agent plus susceptible to this same antimicrobial agent and no resistance gene for this specific antimicrobial agent)

Table 3. Frequency of AMR gene combinations observed in *E. coli* from cattle at slaughter

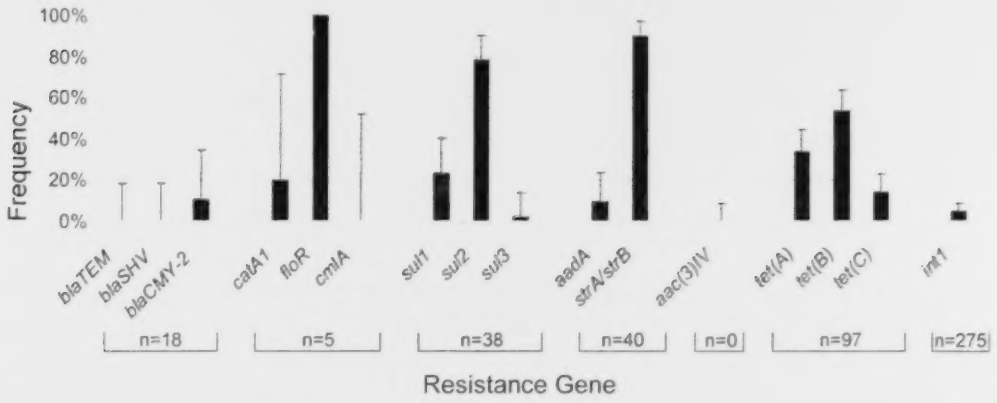
Gene combinations	Frequency
tet(C)	29
sul2-tet(B)-*strA/strB*	19
tet(A)-*strA/strB*	14
tet(A)	13
tet(B)	13
tet(B)-*strA/strB*	9
tet(B)-*tet*(C)-*strA/strB*	5
sul2-tet(B)	4
sul1-aadA	4
sul2-tet(A)-*strA/strB-floR*	2
aadA	1
sul1-sul2-tet(A)-*aadA-catA1-floR*	1
sul1-sul2-tet(A)-bla_{CMY-2}-*aadA-strA/strB-floR*	1
sul1-sul2-tet(A)-*tet*(C)-bla_{CMY-2}-*strA/strB-floR*	1
sul1-tet(B)	1
sul1-tet(B)-*aadA*	1
sul1-tet(C)	1
sul2-tet(B)-*aadA-strA/strB*	1
sul2-tet(B)-*tet*(C)-*strA/strB*	1
sul3-tet(A)-*aadA-cmlA*	1
tet(A)-*aadA*	1
tet(C)-*aadA*	1
tet(C)-*strA/strB*	1
No AMR gene detected	150

Table 4. Statistically significant associations detected between pairs of AMR genes

	bla CMY-2	*catA1*	*floR*	*cmlA*	*sul1*	*sul2*	*sul3*	*aadA*	*strA/strB*	*tet* (A)	*tet* (B)	*tet* (C)
bla CMY-2												
catA1	-											
floR	p=0.000	p=0.018										
cmlA	-	-	-									
sul1	p=0.001	p=0.000	56.35 (6.69-474.66)	-								
sul2	p=0.012	-	p=0.000	-	-							
sul3	-	-	-	p=0.004	-	-						
aadA	-	p=0.044	17.33 (2.45-122.66)	p=0.044	121.33 (15.27-963.90)	-	p=0.044					
strA/strB	p=0.038	-	17.60 (1.83-169.63)	-	-	37.24 (10.64-130.40)	-	-				
tet (A)	p=0.015	-	p=0.000	-	-	-	-	3.88 (1.09-13.87)	6.40 (2.87-14.30)			
tet (B)	-	-	-	-	-	37.24 (10.64-130.40)	-	-	19.58 (8.09-47.43)	p=0.001*		
tet (C)	-	-	-	-	-	-	-	-	-	-	-	
int1	p=0.002	-	35.31 (4.72-263.96)	p=0.051	468.00 (15.52-1.41e+4)	-	-	86.67 (14.02-523.53)	-	12.05 (3.64-39.87)	-	-

Only significant associations are presented. The numbers represent odds ratios for the associations (95% confidence intervals are in parenthesis). -, no significant association detected. *, indicates a negative association. P-values are given for statistically significant positive associations where the calculation of odds ratios was not applicable.

Figure 1. Estimates of AMR genes and *int1* frequencies among *E. coli* isolates resistant to ampicillin, chloramphenicol, sulfonamides, streptomycin, gentamicin, tetracycline, and overall, respectively



Vertical bars above each column represent the upper limit of the 95% confidence intervals on the frequency estimates.

Literature Cited

- Bager F, Aarestrup FM, Madsen M, Wegener HC. Glycopeptide resistance in *Enterococcus faecium* from broilers and pigs following discontinued use of avoparcin. Microb Drug Resist. 1999. 5:53-6.
- Bischoff KM, White DG, Hume ME, Poole TL, Nisbet DJ. The chloramphenicol resistance gene cmlA is disseminated on transferable plasmids that confer multiple-drug resistance in swine *Escherichia coli*. FEMS Microbiol Lett. 2005. 243:285-91.
- Boerlin P, Travis R, Gyles CL, Reid-Smith R, Janecko N, Lim H, Nicholson V, McEwen SA, Friendship R, Archambault M. Antimicrobial resistance and virulence genes of *Escherichia coli* isolates from swine in Ontario. Appl Environ Microbiol. 2005. 71:6753-61.
- Government of Canada. Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) 2003. Guelph, ON: Public Health Agency of Canada, 2005.
- Government of Canada. Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) 2005. Guelph, ON: Public Health Agency of Canada, 2007.
- Gow SP, Waldner CL, Harel J, Boerlin P. Associations between antimicrobial resistance genes in fecal generic *Escherichia coli* isolates from cow-calf herds in western Canada. Appl Environ Microbiol. 2008. 74:3658-66.
- Guerra B, Junker E, Schroeter A, Malorny B, Lehmann S, Helmuth R. Phenotypic and genotypic characterization of antimicrobial resistance in German *Escherichia coli* isolates from cattle, swine and poultry. J Antimicrob Chemother. 2003. 52:489-92.
- Maynard C, Fairbrother JM, Bekal S, Sanschagrin F, Levesque RC, Brousseau R, Masson L, Larivière S, Harel J. Antimicrobial resistance genes in enterotoxigenic *Escherichia coli* O149:K91 isolates obtained over a 23-year period from pigs. Antimicrob Agents Chemother. 2003. 47:3214-21.
- Rowe-Magnus DA, Mazel D. The role of integrons in antibiotic resistance gene capture. Int J Med Microbiol. 2002. 292:115-25.
- Sunde M, Norström M. The genetic background for streptomycin resistance in *Escherichia coli* influences the distribution of MICs. J Antimicrob Chemother. 2005. 56:87-90.
- Tragesser LA, Wittum TE, Funk JA, Winokur PL, Rajala-Schultz PJ. Association between ceftiofur use and isolation of *Escherichia coli* with reduced susceptibility to ceftriaxone from fecal samples of dairy cows. Am J Vet Res. 2006. 67:1696-700.
- Travis RM, Gyles CL, Reid-Smith R, Poppe C, McEwen SA, Friendship R, Janecko N, Boerlin P. Chloramphenicol and kanamycin resistance among porcine *Escherichia coli* in Ontario. J Antimicrob Chemother. 2006. 58:173-7.
- Winokur PL, Vonstein DL, Hoffman LJ, Uhlenhopp EK, Doern GV. Evidence for transfer of CMY-2 AmpC beta-lactamase plasmids between *Escherichia coli* and *Salmonella* isolates from food animals and humans. Antimicrob Agents Chemother. 2001. 45:2716-22.

Other:

Results Communications

A manuscript with the results of this study for publication in an international peer-reviewed journal is in preparation and a copy of the final paper will be forwarded to the *Saskatchewan Agriculture Agricultural Development Fund upon publication.*

The following poster was presented recently at an international meeting on AMR, using the preliminary results of the study:

Hillyer E., P. Boerlin, R. Reid-Smith, A. Deckert, and S. Parker. Distribution of antimicrobial resistance genes and class 1 integrons in generic *Escherichia coli* from Canadian beef cattle. ASM conference on antimicrobial resistance in zoonotic bacteria and foodborne pathogens. Copenhagen, Denmark, June 15-18th 2008. Acknowledgement of the support of Saskatchewan Agriculture Agricultural Development Fund was included in the poster.

High Quality Personnel training

This study is part of the MSc research program of Elizabeth Hillyer at the University of Guelph and was also partially funded by funds of the laboratory of Patrick Boerlin, Department of Pathobiology, Ontario Veterinary College, Guelph.

Expense Statement

(Preliminary estimate pending final account reconciliation)